Human health risk assessment with spatial analysis: Study of a population chronically exposed to arsenic through drinking water from Argentina

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HIGHLIGHTS

• Risk assessment (RA) to As using deterministic procedures
• Integration of RA through deterministic procedures with GIS tools
• Analysis of the time-space behavior of the risk area
• Analysis of As effect outcomes through HI
• Broaden the scopes of deterministic approaches

ARTICLE INFO

Article history:
Received 29 June 2014
Received in revised form 18 August 2014
Accepted 18 August 2014
Available online xxxx

Editor: Damia Barcelo

Keywords:
Arsenic
Water pollution
Public health
Risk assessment
Geographic information systems

ABSTRACT

Arsenic (As) is a ubiquitous element widely distributed in the environment. This metalloid has proven carcinogenic action in man. The aim of this work was to assess the health risk related to As exposure through drinking water in an Argentinean population, applying spatial analytical techniques in addition to conventional approaches. The study involved 650 inhabitants from Chaco and Santiago del Estero provinces. Arsenic in drinking water (Asw) and urine (UAs) was measured by hydride generation atomic absorption spectrophotometry. Average daily dose (ADD), hazard quotient (HQ), and carcinogenic risk (CR) were estimated, geo-referenced and integrated with demographical data by a health composite index (HI) applying geographic information system (GIS) analysis. Asw covered a wide range of concentration: from non-detectable (ND) to 2000 μg/L. More than 90% of the population was exposed to As, with UAs levels above the intervention level of 100 μg/g creatinine.

GIS analysis described an expected level of exposure lower than the observed, indicating possible additional source/s of exposure to inorganic arsenic.

In 68% of the locations, the population had a HQ greater than 1, and the CR ranged between 5·10⁻⁵ and 2,1·10⁻². An environmental exposure area through ADD geo-referencing defined a baseline scenario for space-time risk assessment. The time of residence, the demographic density and the potential health considered outcomes helped characterize the health risk in the region. The geospatial analysis contributed to delimitate and analyze the change tendencies of risk in the region, broadening the scopes of the results for a decision-making process.

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1. Introduction

Arsenic (As) is a ubiquitous element widely distributed in the environment.

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It is transferred from geologic storages to water resources. Consequently, population consuming As contaminated water is chronically exposed to this element.

In Argentina, elevated levels of As in drinking water have been reported since the early twentieth century, being recognized as an endemic region for Hidroarsenicismo Crónico Regional Endémico (HACRE) (WHO, 2012). Currently, As in drinking water has been reported with maximum levels near 200 μg/L in Santa Fe (Vázquez et al., 2000), Catamarca (Bocanegra et al., 2002), Chaco (Blanes et al., 2004), Salta...
Other reports from the provinces of Cordoba (Penedo and Zigaran, 1998), La Pampa (Smedley and Kinniburgh, 2002), Chaco and Santiago del Estero (Bundschu et al., 2004; MSAL, 2007; Navoni et al., 2006; Smedley and Kinniburgh, 2002) have described higher levels, up to 1 mg/L, hundred folds higher than the international guideline level of 10 μg/L (WHO, 2012).

HACRE or arsenosiosis is characterized by a sequence of non-carcinogenic effects such as hyperhidrosis, hyperkeratosis and melanoderma (MSAL, 2001) and a carcinogenic stage that includes skin, bladder, lung, kidney and/or liver cancers (Hopenhayn-Rich et al., 1998; IARC, 2004).

Other pathologies also related to As exposure include bronchitis, chronic obstructive pulmonary disease, bronchiecetasis (Smith et al., 1998, 2006), gangrene, hypertension, peripheral vascular disease, cardiac disorders (Astolfi et al., 1982; Hopenhayn-Rich et al., 1996, 1998; Yuan et al., 2007), non-cirrhotic portal fibrosis and peripheral polyneuropathy. Moreover, diabetes mellitus, mental disorder and cognitive development alterations have also been reported to be associated with arsenic exposure (Calderon et al., 2001; Coronado-Gonzalez et al., 2007; Rocha-Amador et al., 2007; Smith et al., 2006; Yuan et al., 2007).

Despite the knowledge of HACRE as a potential health concern in Argentina, little is known about the health risk associated to the consumption of As through contaminated drinking water of the population.

The use of standardized approaches to conduct risk assessment may be insufficient to perform a comprehensive analysis of the risk scenario.

The inclusion of geographic information system (GIS) analysis broadens the scopes in risk assessment process using proximity analysis of contaminant source as a surrogate for exposure, and integrating environmental monitoring data into the analysis of the health outcomes (Nuckols et al., 2004).

The aim of the present study was to perform a more comprehensive risk assessment by adding spatial analysis to the standard exposure assessment.

In this framework, the specific objectives were: 1) to map water and urine samples for spatial pattern analysis; 2) to calculate the average daily dose intake to demarcate areas of exposure in the studied region and 3) to characterize different risk scenarios taking into consideration the time of residence and the distribution of the population.

2. Materials and methods

2.1. Area of study

The study was performed in an area from the North-center region of Argentina in Santiago del Estero province (Banda and Copo departments) and Chaco province (Almirante Brown department). The study included 19 different locations. Three in Banda department: Jumi Pozo (JP), Negra Muerta (NM), and Siete Árboles (7A); 8 in Copo department: San José del Boquerón (SJB), Uruataí (U), Monte Quemado (MQ), Santos Lugares (SL), Venado Solo (VS), La Firmeza (LF), Malvinas (M), and Las Termas (LT), and 8 in Almirante Brown department: Taco Pozo (TP), Santa Teresa de Cardallo (STC), Pozo Hondo (PH), El Rosillo (ER), San Telmo (ST), Brasil (BR), El Quinto (EQ), and Kilometro 27 (Km27).

Santiago del Estero and Chaco provinces have a total population of 874,006 and 1,055,259 inhabitants, respectively (INDEC, 2010). Fifty four percent of the population is rural and spread and 46% is settled in urbanized centers. Positive intercensal variation has been observed in the last decade indicating a constant increment in demographic density (17.2% A. Brown; 16.4% Copo; 10.8% Banda). The main economic activities are the production of wood, cotton and tobacco, in addition to livestock, farm animal and horticulture production.

The region is an arid and dry zone with a rainy and a dry season. The former has elevated temperatures and a high rate of rainfall. The dry season is represented by a critical (low) humidity level of the soil (Servicio meteorológico Nacional, 2013).

2.2. Population

The population consisted in 650 individuals, aged between 1 and 96 years old.

A survey, following a standard questionnaire, was conducted aiming to collect information about customs, dietary habits and demographic data such as age, sex, years of residence and drinking water patterns. The study was performed in accordance with the Ethical Committee of the Hospital de Clinicas, Buenos Aires, Argentina.

All the objectives of the research were fully explained and informed consent was obtained from all participants.

2.3. Sample collection

Sample collection was performed during the period 2010–2011. Water samples (500 mL) were collected in cleaned plastic bottles containing concentrated nitric acid (HNO3) (final HNO3 concentration 0.015% v/v) and stored at 4 °C until arsenic quantification (Standard Methods, 1998). Different types of water sources were included: tap water (piped water supply), and well water and other storage devices for rainwater.

Urine samples (first void) were collected in polyethylene flasks, previously soaked in 20% v/v HNO3, rinsed with distilled water and dried. No conservatives were used and the samples were kept frozen at −20 °C until analysis. A total of 464 samples were obtained. Urine samples were tested for altered biochemical parameters by urine dipstick analysis. A urinary creatinine concentration between 0.3 and 3.0 g/L was also required as sample inclusion criteria (ACGIH, 2009). Twelve urine samples (2.6%) did not meet the inclusion criteria.

Water samples were geo-referenced through their respective geographic coordinates. Water sample collection was performed house-to-house. Urine samples were grouped according to the location where they were collected.

Arsenic concentration was determined in 192 drinking water samples and, according to the inclusion criteria, in 452 urine samples.

2.4. Sample analysis

Arsenic content was quantified in water and urine samples using a flow injection hydride generation atomic absorption spectrophotometric method. The equipment used was an atomic absorption spectrophotometer (Varian 475) with a hydride generator (Varian VGAA77) and a manual injector (Rhodyne 7125). Briefly, the method is based on the formation of derivatives of arsenic complexes with cysteine. Then, arsines are generated by reaction with sodium borohydride and hydrochloric acid and carried by a nitrogen stream to a heated quartz cell for their atomization and quantitation by atomic absorption spectrophotometry (Navoni et al., 2009, 2010).

The performance of the quantitative procedure was evaluated, for water samples, by the analysis of a reference material: EP-H-2 (EnviromAT Drinking Water) and for urine quantitative analysis, by the use of quality control Lyphochek® level 2 (Biorad). The limit of detection (LOD) and the limit of quantitation (LOQ) of the method were 0.7 and 1.2 μg/L, respectively.

Creatinine was performed by the Enzymatic Assay Kit Method (Wiener lab®). Urinary As (UAs) was expressed as μg of As per g of urine creatinine.

2.5. Exposure assessment

Levels of As in water and urine were evaluated to assess human exposure.
2.6. Human health risk assessment using geographic information system (GIS)

A three steps protocol was followed to assess human health risk.

2.6.1. Hazard identification

Hazard was represented by the levels of As found in drinking water. Expected levels for As in water were calculated from the geo-location of the observed As levels in water, by inverse distance weighted (IDW) interpolation.

2.6.2. Exposure assessment

Exposure was represented by the levels of UAs. The expected UAs levels for the geo-referenced area were obtained by interpolation of the expected levels of As in water, on the urine/water correlation curve.

2.6.3. Risk characterization

The risk was represented by the hazard quotient and the cancer risk. The expected levels of As in water were used to calculate the As average daily dose (ADD) according to the following formula.

\[
ADD = C \times IR \times ED \times EF / BW \times AT
\]

where C represents the expected concentration of As in water (µg/L), IR is the water ingestion rate of 1 and 2 L/day (for less than thirteen years old and older than this age, respectively), ED is the exposure duration (daily fraction), in this case considered = 1, EF is the exposure frequency (365 days/year), BW is the body weight (for people under 13 years old, the weight of the fifty percentile of the corresponding WHO age-weight curve was considered (WHO, 2006), and for 13 years old and older 70 kg) and AT is the average life time span (25,550 days) (US EPA, 1998). ADD was expressed in mg/kg/day.

The group stratification for the GIS estimation of ADD was: 0–1, 0–5, 0–14, 0–20 and 0–40 years old (equivalent to years of residence in the area).

Hazard quotient (HQ) was estimated through the following equation:

\[
HQ = ADD / RID
\]

where RID is the As toxicity reference dose of 0.0003 mg/kg/day for dermatological manifestations (hyperpigmentation and keratosis) (US EPA, 1998).

Health risk situation was assumed when HQ levels were > 1 (Khan et al., 2008).

Cancer risk (CR) was calculated using the formula: \( CR = ADD \times CSF \times IR \times EF / BW \times AT \), where CSF is the cancer slope factor for As of 1.5 mg/kg/day (US EPA, 1998).

A health composite index (HI) was calculated to integrate the information of CR, taking into account the distribution of the population. The HI comes from a polynomial equation that includes many indicators, each one with its respective relative weight. The formula is as follows

\[
HI = (aZ_1 + \cdots + bZ_n) / \sqrt{m - 1}
\]

where \( a \) and \( b \) denote the relative weight of each indicator \( Z \) and the signs + or − indicate the relative contribution; while \( Z \) values are calculated by estimating mean values and standard deviations of each indicator (in this case CR and population) according to the relation

\[
Z = (x_i - \bar{x}) / \sigma
\]

where \( x_i \) is the value of the indicator at each censal radius, \( \bar{x} \) the mean value and \( \sigma \) the standard deviation (PAHO, 2003). CR was derived as described above while the spatial distribution of each age group was estimated on each censal radius (INDEC, 2001) as a proportion of the total population. The contribution of each indicator to the definition of risk was performed separately, giving more weight to the presence of population (75%) than to the concentration of As (25%). The contribution of each indicator to the definition of risk was performed separately, giving more weight to the presence of population (75%) than to the concentration of As (25%). The contribution of each indicator to the definition of risk was performed separately.

Material and methods for risk characterization are summarized in Scheme 1.

3. Results

3.1. Hazard identification

Arsenic level in drinking water was selected as the indicator of hazard. This indicator was linked with its respective water collection system along with the location and the years of residence of the population. All these information is shown in Table 1.

The As level in drinking water showed a wide range of concentrations, from ND up to 2055 µg/L in Pozo Hondo (Chaco province). Banda department was the region with the lowest level of As in drinking water (ND to 63 µg/L). Ninety four percent of the samples presented levels below the current Argentinean guideline level, but only two samples (12%) presented levels below the WHO provisional guideline level of 10 µg/L (WHO, 2012).

Time of residence in the area was also highly variable, from less than a year to almost a century, with averages between one and four decades indicating the long-term exposure of the population to As.

3.2. Exposure assessment

Exposure assessment was performed through the evaluation of UAs. Urinary As levels covered a wide range of concentrations, from 11 to 5085 µg/L (WHO, 2012). Ninety four percent of the samples presented levels above the WHO intervention level of 100 µg/g creatinine.

A statistically significant correlation was observed between UAs level and As in drinking water \( Y = 1.5x + 233.5 \) (Pearson's correlation coefficient: 0.81; \( P < 0.00001 \)).

Fig. 1 describes the spatial variation estimated for As in drinking water (circles) and for UAs level (surface). In both representations the size of the circles and the gray tone graduation represent As concentration in water and urine, respectively.

Despite being larger the number of urine samples than the number of water samples, fewer dots represent the distribution of urine samples, because the schools, where the collection of urine samples was centralized, and as a geographic reference.

In Fig. 2, the spatial distribution of the urinary levels using the correlation equation is described. Black bars represent the UAs levels (expected levels) estimated by the equation. White and gray bars represent the maximum and the mean UAs levels (observed levels) by location, respectively.
In most of the cases, the observed level was higher than the expected level. MQ and ER presented an expected UAs higher than the observed, a fact that could be explained by the possibility of the exposure to other additional sources of inorganic arsenic, such as food or dust and the availability of water with low As levels such as the use of rainfall water as drinking water respectively.

### 3.3. Risk characterization

The ADDs estimated for people resident in the area were ranged between 0.3 and 138.0 $\times 10^{-4}$ mg/kg/day. Sixty eight percent of the population had a HQ greater than 1. The CR was between $2 \times 10^{-2}$ and considering $10^{-4}$ as a limit CR (EPA, 2005), 74% of the population had an increased risk for lung and bladder cancers.

In Fig. 3 the HQ is related to the time of residence. The changes that occur over time are represented by the observed variations in the extension of zones in risk.

An increasing map of health risk could be observed over the years, as the residence time increases.

Population that have lived all their lives in the region and have an age of 40 years or older are in risk situation for As related dermal lesions regardless of where they live or have lived.

A health composite index (HI) was calculated by age group in order to estimate cancer risk (CR). The age range selected (0–14 years old

### Table 1

<table>
<thead>
<tr>
<th>Department</th>
<th>Water collection/distribution system</th>
<th>Population</th>
<th>Time of residence (years)</th>
<th>As in drinking water ($\mu$g/L)</th>
</tr>
</thead>
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<tr>
<td></td>
<td>Location</td>
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<td>Mean</td>
<td>Range</td>
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<td></td>
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<td>14</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>Well</td>
<td>M</td>
<td>5</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>Cistern/well</td>
<td>LT</td>
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<td>26</td>
</tr>
<tr>
<td></td>
<td>Cistern/well</td>
<td>LF</td>
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<td>39</td>
</tr>
<tr>
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<td>Well</td>
<td>SL</td>
<td>19</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>Well</td>
<td>VS</td>
<td>28</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>Well</td>
<td>SJB</td>
<td>95</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Pipe water supply</td>
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<td>75</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>Well</td>
<td>ST</td>
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<td>Well</td>
<td>BR</td>
<td>20</td>
<td>12</td>
</tr>
<tr>
<td></td>
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<td>12</td>
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<td>16</td>
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<td>Well</td>
<td>EQ</td>
<td>34</td>
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</tr>
<tr>
<td></td>
<td>Cistern/well</td>
<td>PH</td>
<td>20</td>
<td>34</td>
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</tbody>
</table>

**Scheme 1.** Flow chart of experimental design for risk characterization.
(young population) and 0–40 years old (adult population)) illustrates short and long exposure periods of the population (Fig. 4).

The probability that a resident becomes ill as a result of consuming As through drinking water was estimated through the CR and it is represented in Fig. 4a. Fig. 4b shows the distribution (density) of the population in age groups, expressed as a proportion of the total population and represented in percentiles.

The probability for the population living for 14 and 40 years in a specific area to develop cancer is represented by the HI (Fig. 4c).

Places were characterized according to water arsenic concentration and population density in order to arrange them in a scale of risk. The risk area increased according to the time of residence in the same way that HQ did. Considering the first and third tertiles (the lowest and the highest risk population groups), the population involved was 5965 and 7558 inhabitants, for the group aged 0–14, and 21,803 and 29,767 inhabitants, for the group aged 0–40, respectively. Analyzing this information, an 1.39 and 1.53 folds increase (respectively) of the relative risk of cancer for each group was established. This observation indicates that according to the model applied, the risk for being exposed to arsenic in the first years of life would be almost the same as that for longer periods of exposure.

### Table 2

<table>
<thead>
<tr>
<th>Location</th>
<th>Population</th>
<th>UAs (μg/g creat.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Mean</td>
</tr>
<tr>
<td>MQ</td>
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<td>LF</td>
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<td>ST</td>
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<td>482</td>
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<td>ER</td>
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<td>577</td>
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<tr>
<td>VS</td>
<td>16</td>
<td>2190</td>
</tr>
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</table>

The presence of high levels of As in drinking water has been described since the early twentieth century, along with the relationship with its deleterious effects. There are numerous reports (Penedo and Zigaran, 1998; Vázquez et al., 2000; Bocanegra et al., 2002; Smedley and Kinniburgh, 2002; Blanes et al., 2004; Bundschuh et al., 2004;
Concha et al., 2006; Navoni et al., 2006, 2012; MSAL, 2007; Guber et al., 2009) highlighting the current situation of geogenic As contamination in different regions of Argentina.

In this study, high levels of As, with maximum levels up to 200 times the recommended international standard of 10 μg/L (WHO, 2012), were found. Contamination showed to be persistent over the time, according to data previously reported (Concha et al., 1998; Navoni et al., 2006; MSAL, 2007). The levels of As found in water were comparable to those reported for other countries with endemic regions of HACRE such as, Chile, México, India and Bangladesh (Tondel et al., 1999; Ng et al., 2003; Smith and Smith, 2004; Rahman et al., 2005).

The lack of safe water sources places the population (especially those individuals who live isolated or in small locations in rural areas) against long periods of drought and, as a result, leads them to consume water with high levels of As.

Mainly at the rural areas, people used to consume water from different sources, such as groundwater or rain water collected into reservoirs (cisterns) during the rainy season. Urban population used to consume almost exclusively water from the distribution systems. Water from reservoir systems was not expected to have As, because it usually comes from rain. However, variable (sometimes high) As levels were found.

Moreover, removal techniques (rarely available) did not guarantee the provision of safe water.

Monte Quemado and Urutú water supply comes from a narrow aqueduct, called Canal de Dios (God’s Channel), with an As level near to 1 mg/L. The As content is reduced to acceptable levels (10 μg/L) by a treatment based on the precipitation/floculation of arsenic with ferrous chloride, in a treatment plant installed 50 years ago.

Unlikely, Taco Pozo applies a treatment to reduce the As content based on the precipitation/floculation with aluminum salts but it does not reach the desired concentration of 10 μg/L.

The urine is the best biological specimen for assessing recent exposure to As (ATSDR, 2007). In the same way that occurred with As in drinking water, the levels of UAs found in this population showed an exposure comparable with those found in other areas from Latin America such as Chile (Biggs et al., 1997; Moore et al., 1997), México (Hernández-Zavala et al., 1998; McClintock et al., 2012) and quite similar to those reported for India and Bangladesh (Samanta et al., 1999; Basu et al., 2005).

After analyzing the results of As in water and UAs levels by GIS, a new and more comprehensive risk scenario was obtained. The new scenario allowed us to better characterize the region and to identify areas of potential health risk. Despite the broadening of the health risk information provided by the application of the GIS tools, some differences between observed and estimated UAs levels were observed. These differences could be explained by the existence of an additional source of exposure to inorganic As, such as food or dust (Navoni et al., 2007; Uchino et al., 2006). However, a new analysis including these other additional sources of exposure should be performed to confirm this hypothesis.

The geographical pattern that supports a probable association between environmental contaminants and health effects could be affected by differences in the data collection method used (Nieuwenhuijsen et al., 2006; Jarup, 2004; Beyea and Hatch, 1999). In the case of this work the results of the interpolation was more precise around urban areas than in rural areas due to the higher availability of data of the former.

Another factor that could affect the estimation of the exposure is the latency time. Questionnaire information could not reflect reliably the behavioral changes in exposure conditions that took place in the past and, therefore, the estimation of the exposure could have some bias (explaining why the observed UAs levels in the most of cases were higher than the expected UAs levels).

![Fig. 2. Comparison of the expected urinary arsenic level (UAs) with mean and maximum levels found in people living in the study sites.](image-url)
The application of GIS analysis to risk assessment resulted in a more and better, based-in-evidence, prediction of the exposure in areas not studied yet. It also helped to predict exposure levels in non-evaluated population.

Skin is considered the most sensitive organ to As exposure. Mosaferi et al. (2007) found a clear dose–response relationship between skin disorders and As in drinking water below 150 μg/L. People from all the locations in Almirante Brown department and more than a half of the places studied from Copo department may thus be expected to develop dermatological manifestations.

The HQ for dermal effects calculated in this work, described a risk area dependent not only to the level of As in water but also of the time of exposure (years of residence). Thus, the distance to a particular point in relation with the source of exposure represented an estimation of the risk.

Skin tumors are the carcinogenic effect most related to long-term As exposure (Karagas et al., 2000; Chen et al., 2005; Mosaferi et al., 2007; Kazi et al., 2009). The transformation of skin lesions into skin tumors is considered a warning sign for the development of cancers of internal organs (Cowlishaw et al., 1979; Tello, 1986; Bates et al., 1992). Based on the results of this work, a high rate of dermal lesions and internal tumors is expected in the studied population.

The inclusion of the time of residence in the GIS analysis helped predict the risk scenario over time. This prediction would be an important contribution of evidence, as populations of low socioeconomic status are neither subject of risk planning programs nor of a warning criterion of hazard management.

Compared to CR, HI included the distribution of the population and gave a more reliable measure of the cancer risk integrating the hazard (As in water) and the vulnerability (described as the proportion of people living in a particular area and consuming As-contaminated water).

In this way, HI becomes a better indicator of health risk, due to the combination of several indicators that allow assessing the risk over time on complex issues.

5. Conclusion

As stated before, the objective of this work was to perform a risk assessment by applying spatial analytical approaches. An improvement in the scope of the health risk assessment, due to exposure to As, was possible by the integration of standardized procedures with demographic information through GIS analysis.

In addition to the hazard inference in relation to the presence of high levels of inorganic As in a large area of two Argentine provinces, the re-conceptualization of the exposure as a time and space dependent variable, led to redefine the risk status of the population in the studied region.

The information obtained displays future scenarios to be studied helping to define priorities for risk management (implementation of mitigation procedures in particular areas, the incorporation of toxicological monitoring programs and/or epidemiological assessment) in an area of centennial history of hydroarsenicism.

References

**Fig. 4.** Carcinogenic risk (CR) (a), population density (%) (b) and calculated health composite index (HI) (c) distributions, according to two cross-cuts (0–14 and 0–40).


Pob. (0-40 / tot)*100

<table>
<thead>
<tr>
<th>Tercil</th>
<th>0 - 50</th>
<th>51 - 100</th>
<th>101 - 150</th>
<th>151 - 200</th>
<th>201 - 250</th>
<th>251 - 300</th>
<th>301 - 350</th>
<th>351 - 400</th>
<th>401 - 450</th>
<th>451 - 500</th>
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HI (tercil)

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<tr>
<th>Tercil</th>
<th>-1.1 - 0.3</th>
<th>-0.2 - 0.1</th>
<th>0.0 - 3.7</th>
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</thead>
<tbody>
<tr>
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<td>21 - 38</td>
<td>39 - 42</td>
<td>43 - 53</td>
</tr>
<tr>
<td>Pob. (40-0 / tot)*100</td>
<td>50 - 70</td>
<td>71 - 74</td>
<td>75 - 88</td>
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<tr>
<td>HI (tercil)</td>
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<td>-0.3 - 0.3</td>
<td>0.4 - 3.1</td>
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